ZooKeys 1040: 187–200 (2021)
doi: 10.3897/zookeys.1040.66527
https://zookeys.pensoft.net

RESEARCH ARTICLE

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New or little-known *Boreoheptagyia* (Diptera, Chironomidae) in China inferred from morphology and DNA barcodes

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Academic editor: F.L. da Silva | Received 26 March 2021 | Accepted 7 May 2021 | Published 28 May 2021

http://zoobank.org/F7D0867D-FB49-48CA-B89E-FC27B06474E1

Citation: X-L Lin, Yu H-J, Wang X-H, Bu W-J, Yan C-C, Liu W-B (2021) New or little-known *Boreoheptagyia* (Diptera, Chironomidae) in China inferred from morphology and DNA barcodes. ZooKeys 1040: 187–200. https://doi.org/10.3897/zookeys.1040.66527

Abstract

The male adult of *Boreoheptagyia zhengi* Lin & Liu, **sp. nov.** is described and illustrated based on material collected in China. Associated morphological characteristics and reference to its DNA barcode are provided. *Boreoheptagyia kurobebrevis* (Sasa & Okazawa, 1992) is newly recorded from China based on both a male and female, with additional associated data on the DNA barcode of the male adult. A neighbor-joining tree based on available *Boreoheptagyia* DNA barcodes and a key to the adults of *Boreoheptagyia* from China are given.

Keywords

COI, Diamesinae, integrative taxonomy, new species, non-biting midges

Introduction

Brundin (1966) erected the genus *Boreoheptagyia* with *Heptagyia rugosa* Saunders, 1930 as type species by original designation. Larval populations of this genus live in cool, fast-flowing streams and other harsh environments (Thienemann 1954; Oliver 1989).

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At present, the genus includes 24 valid species worldwide (Ashe and O'Connor 2009). Among them, 17 are restricted to the Palaearctic Region including the two recently described species *B. joeli* Makarchenko, 2020 and *B. ortladamellica* Rossaro, 2017 (Rossaro 2017; Makarchenko et al. 2020), seven to the Oriental Region and one to the Nearctic Region. Only *B. rotunda* Serra-Tosio, 1983 occurs in both Palaearctic and Oriental regions (Ashe and O'Connor 2009; Rossaro 2017). Seven species are currently reported from China: *B. alulasetosa* Makarchenko, Wu & Wang, 2008, *B. ambigua* Makarchenko, Wu & Wang, 2008, *B. brevitarsis* (Tokunaga, 1936), *B. similis* (Chaudhuri & Ghosh, 1981), *B. tibetica* Makarchenko, Wang & Willassen, 1996, *B. xinglongiensis* Makarchenko, Wu & Wang, 2008 and *B. joeli* Makarchenko, 2020 (Makarchenko et al. 1996; Wang 2000; Makarchenko et al. 2008; Makarchenko et al. 2020).

The DNA barcode corresponding to the 658-bp fragment of the mitochondrial gene cytochrome *c* oxidase I (COI) has been identified as the core of a global bio-identification system at the species level (Hebert et al. 2003a, b) and has proved to be useful in the delimitation of non-biting midge species and has provided important evidence to confirm new species (Anderson et al. 2013; Lin et al. 2015; Giłka et al. 2018; Lin et al. 2018; Song et al. 2018; Lin et al. 2019; Liu et al. 2021).

In the present study, morphology and the DNA barcode of *B. zhengi* Lin & Liu, sp. nov. are provided based on material collected in Yunnan Province, China. *Boreoheptagyia kurobebrevis* (Sasa & Okazawa, 1992) is newly recorded from China based on a male and female, the latter was associated with the male by standard DNA barcodes. DNA barcode analysis including the partial COI sequences of species of genus *Boreoheptagyia* is conducted. A key to the known adults of *Boreoheptagyia* from China is also given.

Materials and methods

The examined adults were preserved in 85% ethanol and stored in the dark at 4 °C before morphological and molecular analyses. Genomic DNA was extracted from the thorax and head using a Qiagen DNA Blood and Tissue Kit at Nankai University, Tianjin, China (**NKU**), following the standard protocol (Lin et al. 2018) except for the final elution volume of 100 μl. After DNA extraction, the exoskeleton of each specimen was mounted in Euparal on a microscope slide together with the corresponding wings, legs, antennae and abdomen, following the procedures outlined by Sæther (1969). Morphological terminology follows Sæther (1980).

Digital photographs of the mounted specimens were taken at 300-dpi resolution using a Nikon Digital Sight DS-Fil camera mounted on Nikon Eclipse 80i compound microscope using the software NIS-Elements F v.4.60.00. at the College of Life Sciences, Nankai University, Tianjin, China (NKU).

The universal primers LCO1490 and HCO2198 (Folmer et al. 1994) were used to amplify the standard 658-bp mitochondrial COI barcode region. Polymerase chain reaction (PCR) amplifications followed Song et al. (2018) and were conducted in a

25 µl volume including 12.5 µl 2× Es Taq MasterMix (CoWin Biotech Co., Beijing, China), 0.625 µl of each primer, 2 µl of template DNA and 9.25 µl of deionized H_2O. PCR products were electrophoresed in 1.0% agarose gel, and purified and sequenced in both directions at Beijing Genomics Institute Co., Ltd., Beijing, China.

Raw sequences were assembled and edited in Geneious Prime 2020 (Biomatters Ltd., Auckland, New Zealand). Alignment of the sequences was carried out using the MUSCLE algorithm (Edgar 2004) on amino acids in MEGA X (Kumar et al. 2018). The pairwise distances using the Kimura 2-Parameter (K2P) substitution model of ten species within the genus *Boreoheptagyia* were calculated in MEGA. The neighbor-joining tree was constructed using the K2P substitution model, 1000 bootstrap replicates and the "pairwise deletion" option for missing data in MEGA. Novel sequence, trace-files, and metadata of the new species are uploaded to the Barcode of Life Data Systems (BOLD) (Ratnasingham and Hebert 2013). GenBank accessions of the Chinese specimens are list in Table 1. The holotype of the new species and other examined specimens are deposited in the collection of the College of Life Sciences, Nankai University, Tianjin, China.

Results

Taxonomic description

***Boreoheptagyia kurobebrevis* (Sasa & Okazawa, 1992)**
Figs 1–4

Diamesa kurobebrevis Sasa & Okazawa, 1992: 58.
Toyamadiamesa kurobebrevis Sasa & Kikuchi, 1995: 205.
Boreoheptagyia kurobebrevis Endo, 2002: 12; Makarchenko et al. 2008: 8; Ashe and O'Connor 2009: 265.

Material examined. Male (NKU & BOLD sample ID: LGS63), 1 male, China, Guizhou Province, Qiandongnan Miao and Dong Autonomous Prefecture, Leishan, Leigongshan Natural Reserve, Fangxiang, Getoucun, 26.396014°N, 108.260933°E, 1070 m a.s.l., Malaise trap, 10–30. i. 2020, leg. H.-J. Yu. 1 female, same as above.

Diagnostic characters. *B. kurobebrevis* can be distinguished from other related species in having: antenna with five flagellomeres; wing membrane covered with microtrichia on entire surface. Costal extension 175 µm long. R with 31 setae, R_1 with 34 setae, R_{4+5} with 31 setae. Superior volsella rounded; inferior volsella finger-like and well-sclerotized; gonostylus with 2 small megasetae.

Adult male (N = 1). Total length 2.95 mm. Wing length 2.60 mm. Total length/wing length 1.13. Wing length/length of profemur 2.17.

Coloration (Fig. 1). Head, thorax and abdomen mostly dark brown. Ground color of scutum yellow, stripes and postnotum dark brown, scutellum brown, abdominal tergites almost uniformly yellow, hypopygium dark brown, femur yellow in basal three-

Table 1. Kimura 2-parameter pairwise genetic distances based on COI barcodes of the *Boreoheptagyia*.

Species	Pairwise genetic distances																						GenBank accessions
Boreoheptagyia zhengi																							MZ128909
Boreoheptagyia brevitarsis	15.1																						MZ128906
Boreoheptagyia kurobebrevis	14.9	14.1																					MZ128908
	14.9	14.1	0.0																				MZ128907
Boreoheptagyia joeli	13.7	11.2	12.9	12.9																			MT240752
	14.3	11.9	13.5	13.5	0.9																		MT240753
	14.1	11.9	13.3	13.3	0.8	1.1																	MT240754
	13.9	11.8	13.1	13.1	0.8	0.5	0.9																MT240755
Boreoheptagyia sarymsactyensis	13.9	13.6	15.9	15.9	11.0	10.8	11.1	10.6															MT240756
	14.1	13.8	16.1	16.1	11.2	11.0	11.3	10.8	0.2														MT240757
	14.3	13.4	16.1	16.1	10.8	10.6	11.0	10.4	0.8	0.6													MT240758
Boreoheptagyia brevitarsis	12.1	11.8	12.9	12.9	9.2	9.9	9.9	9.7	11.7	11.9	12.2												MT240774
Boreoheptagyia sp. EAM-2017	13.2	12.5	13.3	13.3	10.3	10.1	10.4	9.9	7.0	7.2	7.5	11.0											KY640386
Boreoheptagyia alulasetosa	17.1	15.7	16.6	16.6	12.7	13.3	13.1	12.9	14.2	14.4	14.4	14.9	15.1										MZ128904
Boreoheptagyia iranica	15.6	14.9	14.4	14.4	13.3	13.7	14.2	13.7	16.1	16.3	16.3	13.8	14.0	15.7									MT240768
	15.6	14.9	14.4	14.4	13.3	13.7	14.2	13.7	16.1	16.3	16.3	13.8	14.0	15.7	0.0								MT240769
	15.6	14.9	14.4	14.4	13.3	13.7	14.2	13.7	16.1	16.3	16.3	13.8	14.0	15.7	0.0	0.0							MT240770
	15.6	14.9	14.4	14.4	13.3	13.7	14.2	13.7	16.1	16.3	16.3	13.8	14.0	15.7	0.0	0.0	0.0						MT240771
Boreoheptagyia brevitarsis	12.5	12.7	13.9	13.9	10.4	11.1	11.1	11.0	12.4	12.6	12.9	1.2	11.7	14.7	14.2	14.2	14.2	14.2					MT240772
	11.6	12.3	13.7	13.7	9.6	10.1	10.2	9.9	11.5	11.7	12.0	0.8	11.0	14.9	14.0	14.0	14.0	14.0	1.5				MT240773
	14.7	0.5	13.9	13.9	11.0	11.7	11.9	11.5	13.3	13.5	13.1	11.7	12.6	15.7	15.0	15.0	15.0	15.0	12.6	12.2			MZ128905
	11.8	11.9	13.1	13.1	9.6	10.1	10.2	9.9	11.8	12.0	12.4	0.5	10.8	15.3	13.6	13.6	13.6	13.6	1.4	0.8	11.9		MT240775
	11.7	12.3	13.5	13.5	9.9	10.2	10.2	10.1	11.7	11.8	12.2	0.8	11.1	14.9	14.2	14.2	14.2	14.2	1.9	0.9	12.2	1.1	MT240775

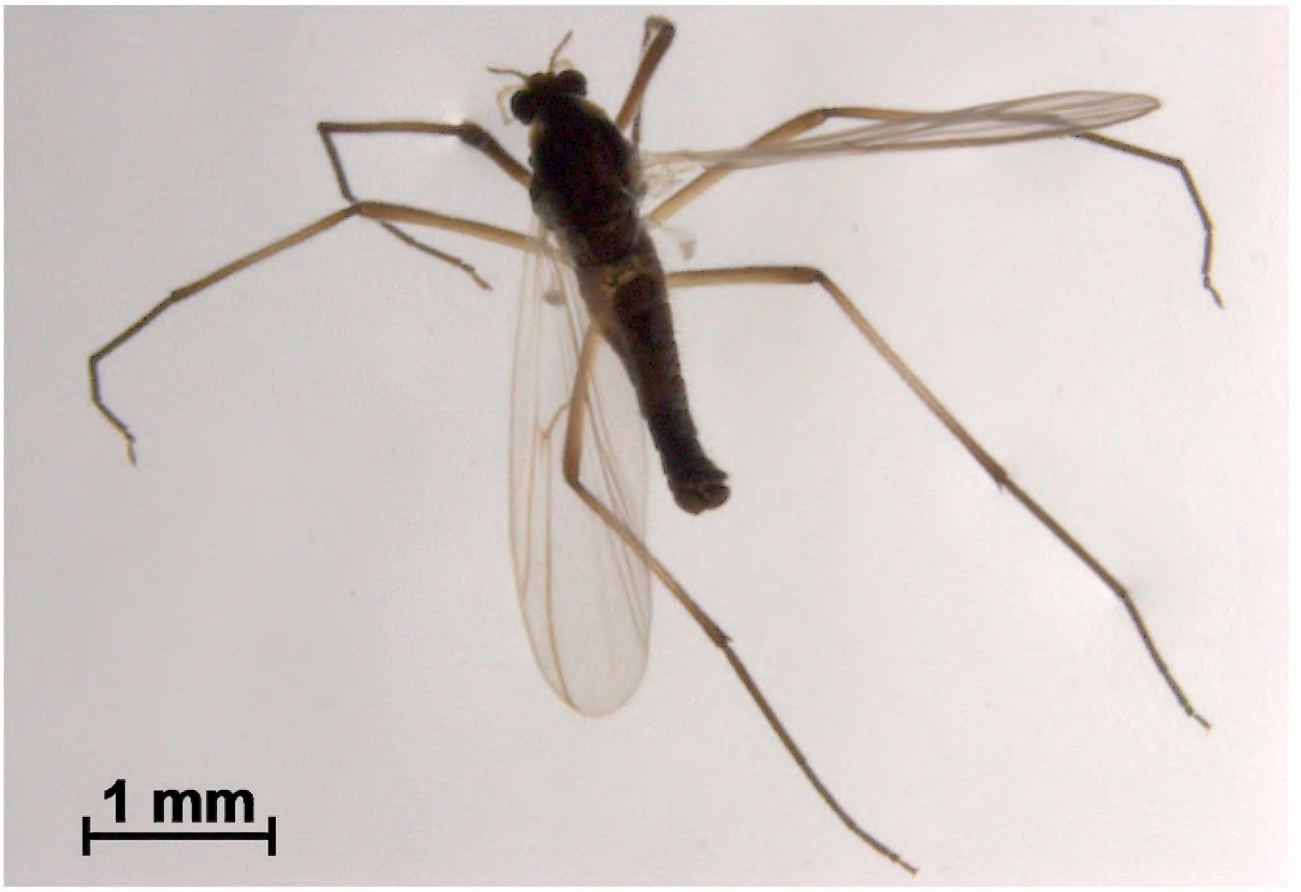

Figure 1. *Boreoheptagyia kurobebrevis* (Sasa & Okazawa, 1992), male adult in ethanol.

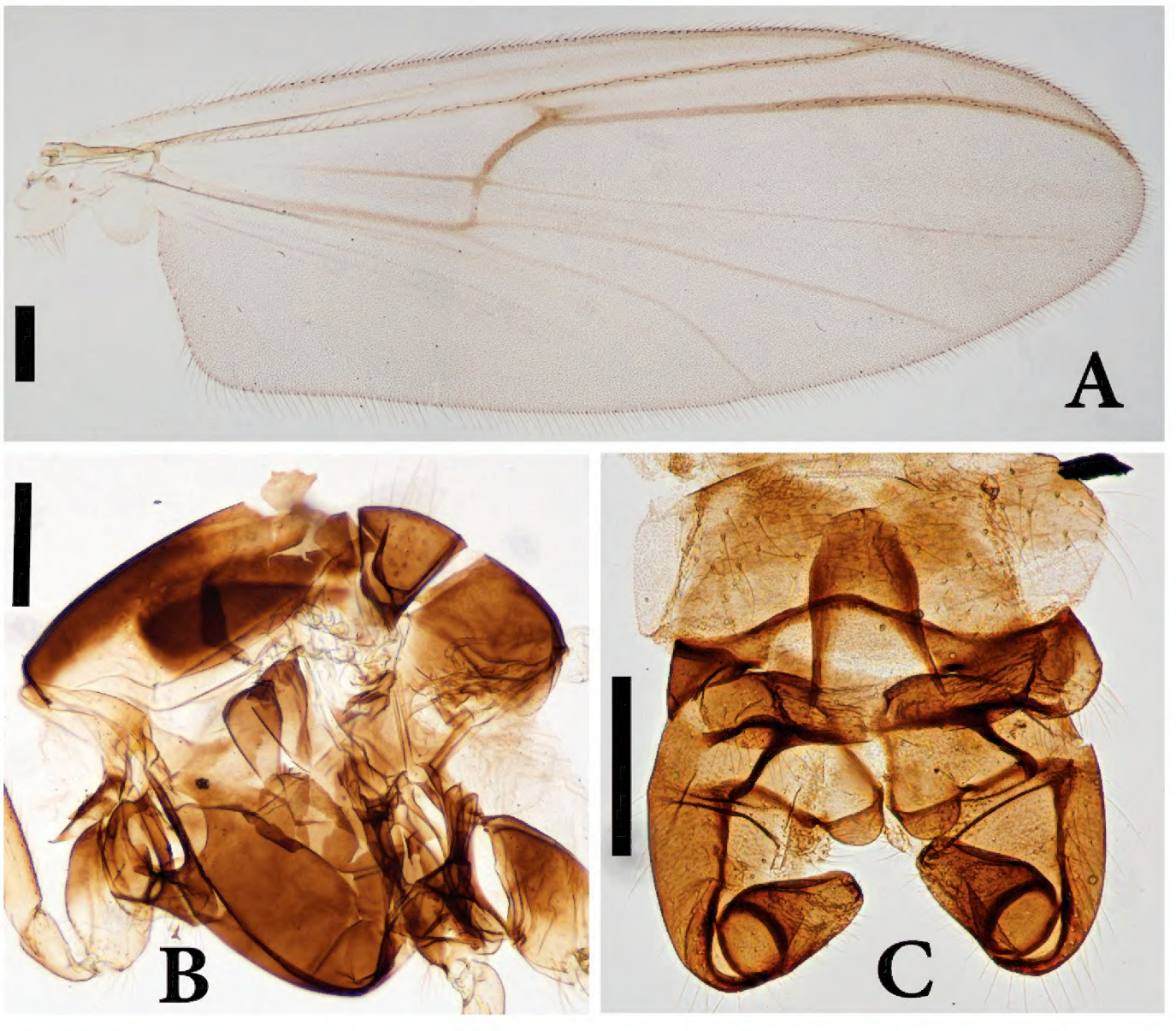

Figure 2. *Boreoheptagyia kurobebrevis* (Sasa & Okazawa, 1992), male adult **A** wing **B** thorax **C** hypopygium. Scale bars: 200 μm.

Figure 3. *Boreoheptagyia kurobebrevis* (Sasa & Okazawa, 1992), female adult.

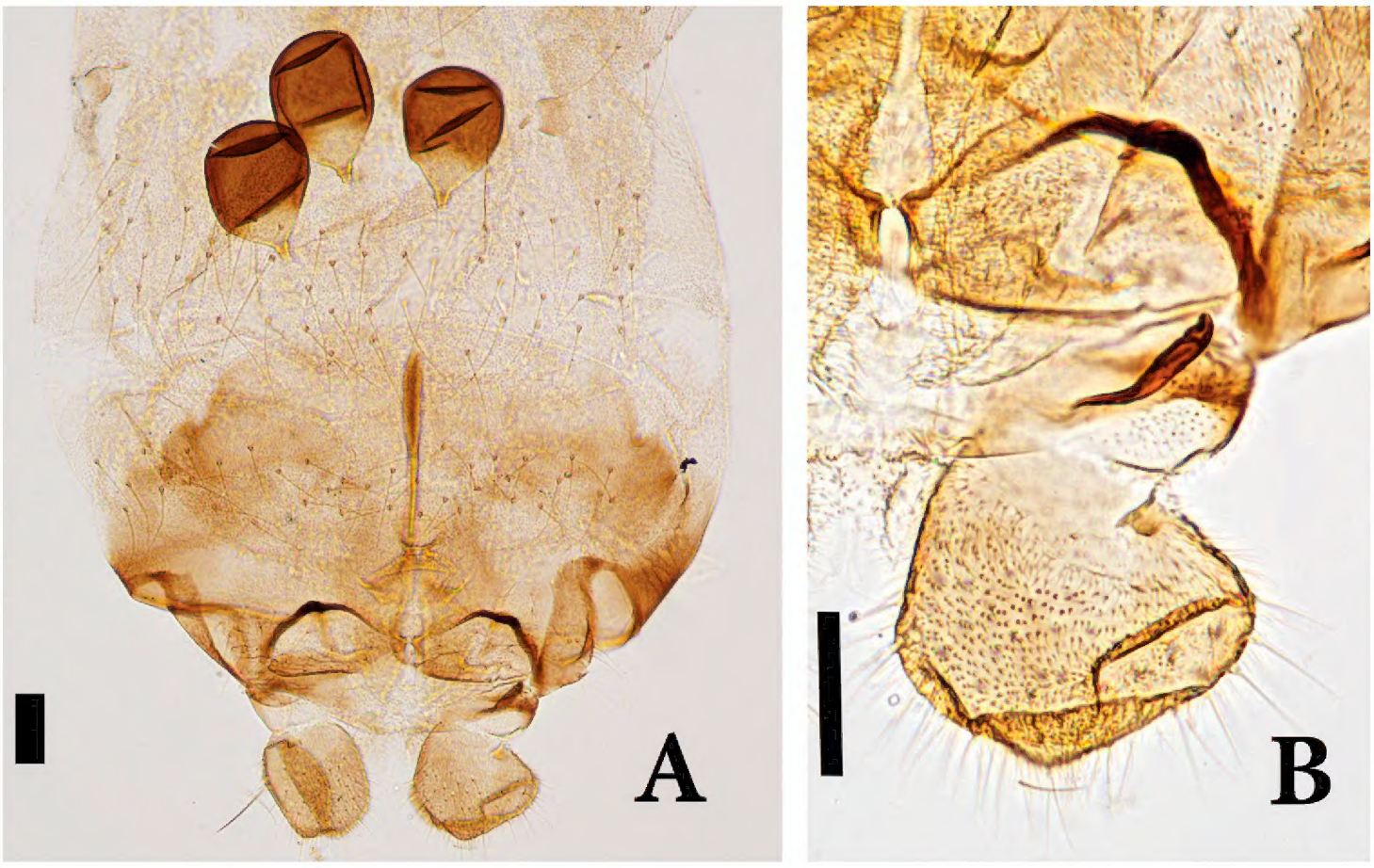

Figure 4. *Boreoheptagyia kurobebrevis* (Sasa & Okazawa, 1992), female adult **A** genitalia with gonapophysis VIII and seminal capsules, ventral **B** cercus. Scale bars: 50 μm.

Table 2. Lengths (in μm) and proportions of legs of *Boreoheptagyia kurobebrevis* (Sasa & Okazawa, 1992) in China, male (*N* = 1).

	fe	ti	ta_1	ta_2	ta_3	ta_4	ta_5	LR	BV	SV	BR
P_1	1200	1380	920	500	270	80	130	0.67	3.57	2.80	2.64
P_2	1300	1220	750	480	240	70	120	0.61	3.59	3.36	3.50
P_3	1260	1450	900	500	250	70	90	0.62	3.97	3.01	3.89

quarter with brown ring apically, tibia pale medially with brown rings in basal one-fifth and distal one-third.

Head. Antenna with five flagellomeres. AR 0.82. Temporal setae 8, not separable into inner and outer verticals. Clypeus with 20 setae. Tentorium 75 μm long; 25 μm wide. Lengths of palpomere 1–5 (in μm): 38, 50, 105, 158, 250. Length ratio of palpomeres 5/3: 2.38.

Thorax (Fig. 2B). Antepronotum with 9 anterolateral setae; acrostichals 28; dorsocentrals 17 in two rows; prealars 10. Scutellum with 46 setae.

Wing (Fig. 2A). Brachiolum with 5 setae; wing membrane with macrotrichia on entire surface; costal extension 175 μm long. Distribution of setae on veins: R, 31; R_1, 34; R_{4+5} 3. Anal lobe well-developed; squama with 16 setae. VR 1.08.

Legs. Length (in μm) of spurs of: P_1, 40; P_2, 55 and 55; P_3, 80 and 50. Width (in μm) of tibial apex of: P_1, 60; P_2, 70 μm; P_3, 100. Comb on hind tibia with Comb of hind tibia with 12 setae. Lengths (in μm) and proportions of legs as in Table 2.

Hypopygium (Fig. 2C). Tergites IX without anal point. Tergites IX with 30 setae.

Gonocoxite 300 μm long. Superior volsella rounded, 60 μm long. Inferior volsella well-sclerotized, finger-like, bearing 17 setae. Gonostylus 140 μm long, with two small megasetae. HR 2.14; HV 2.11.

Genitalia of female (*N* = 1) (Figs 3, 4). *Gonocoxite* IX 100 μm long, 45 μm wide, with 7 setae. Cercus 88 μm long, 75 μm wide, with 47 setae and covered with macrotrichia. Seminal capsule 158 μm long, 75 μm wide, sclerotized part 98 μm long.

***Boreoheptagyia zhengi* Lin & Liu, sp. nov.**

http://zoobank.org/9B5BB346-3355-469A-9F0E-125C350041E7

Figs 5, 6

Type material. ***Holotype*:** male (NKU & BOLD sample ID: XL3519), Yunnan Province, Baoshan City, Mangkuan County, Gaoligongshan National Nature Reserve, Baihualing, near a remote wild hot spring and a stream, 25.3105556°N, 98.795000°E, 1475 m a.s.l., light trap, 22.v.2018, leg. X.-L. Lin.

Diagnostic characters. According to the morphological characters of the adult male, the new species keys to the genus *Boreoheptagyia*. The new species is distinguished from its other congeners by the following combination of characters: antenna with seven flagellomeres; wing membrane covered with microtrichiae on almost entire surface except a bare

Figure 5. *Boreoheptagyia zhengi* Lin & Liu, sp. nov., male adult, holotype.

area near anal lobe; femora and tibiae of all legs pale in basal half, other portions brown; superior volsella tongue shape with small projection; gonostylus with one megaseta.

Adult male (*N* = 1). Total length 2.10 mm. Wing length 1.42 mm. Total length/wing length 1.48. Wing length/length of profemur 1.63.

Coloration (Fig. 5). Head, thorax and abdomen yellow-brown. Wing membrane with microtrichiae on almost entire surface except a bare area near anal lobe. Femur and tibia of all legs pale in basal half, other portions brown.

Head (Fig. 6A, B). Antenna with seven flagellomeres; ultimate flagellomere 55 μm long; AR 0.31. Eyes bare without dorsomedial extension. Temporal setae 14, not separable into inner and outer verticals. Clypeus with nine setae. Lengths of palpomere 1–5 (in μm): 20, 30, 60, 80, 150. Palpomere ratio ($5^{th}/3^{rd}$): 2.50.

Thorax (Fig. 6C). Antepronotum with five setae; acrostichals 27; dorsocentrals 14 in two rows; prealars five. Scutellum with 30 setae.

Wing (Fig. 6D). VR 1.04. Brachiolum with three setae. Costa without extension. Squama with eight setae. R with 11 setae, R_1 with four setae, R_{4+5} with five setae. Anal lobe developed.

Legs. Spur of front tibia 35 μm long, of mid tibia 38 μm long; of hind tibia 55 and 33 μm long. Width of front tibia apex 50 μm, of mid tibia apex 50 μm, of hind tibia apex 63 μm. Comb of hind tibia with 15 setae. Lengths (in μm) and proportions of legs as in Table 3.

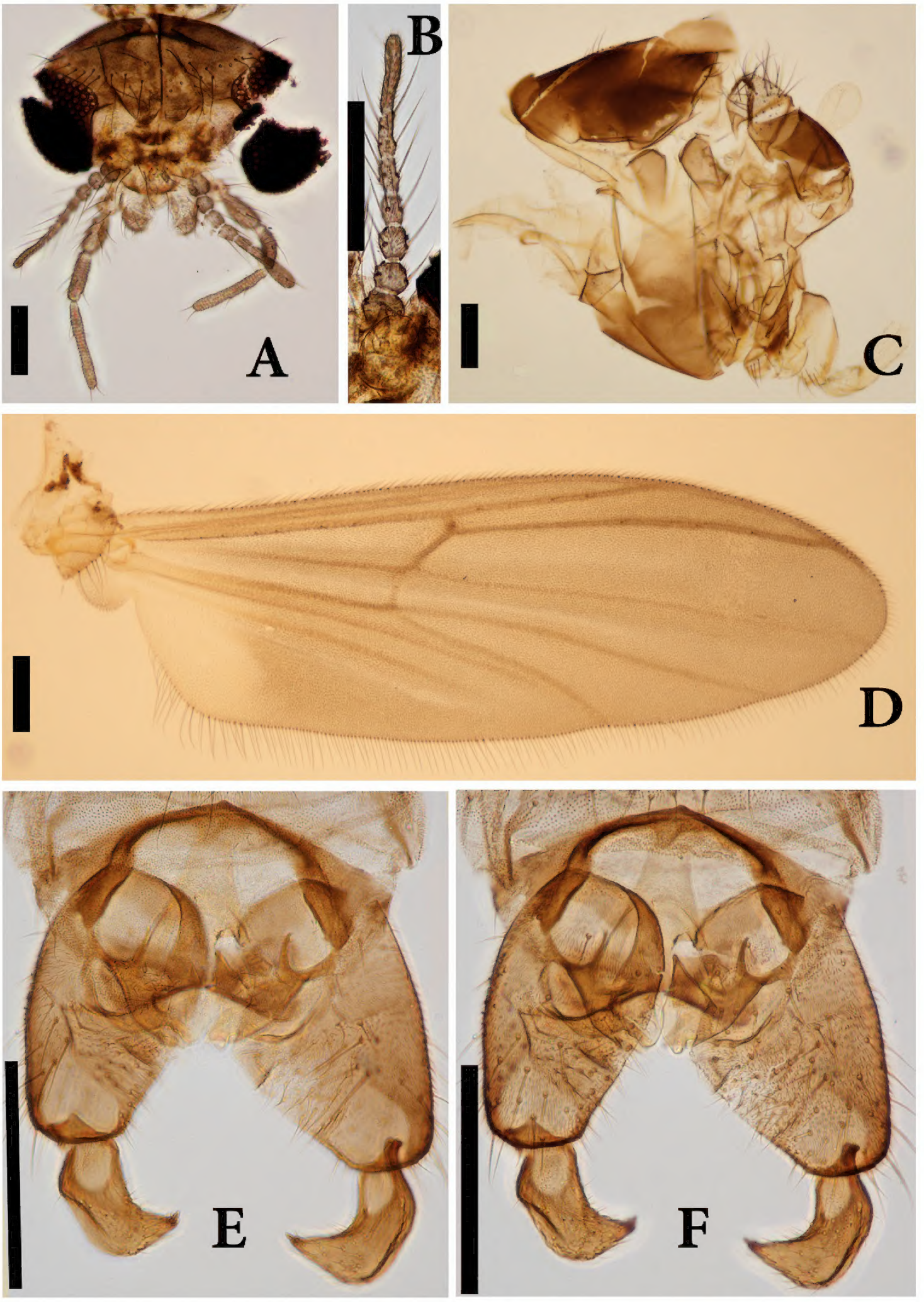

Figure 6. *Boreoheptagyia zhengi* Lin & Liu, sp. nov., male adult, holotype **A** hea **B** antenna **C** thorax **D** wing **E** hypopygium, dorsal view **F** hypopygium, ventral view. Scale bars: 100 μm.

Table 3. Lengths (in μm) and proportions of legs of *Boreoheptagyia zhengi* Lin & Liu, sp. nov., male holotype (*N* = 1).

	fe	ti	ta_1	ta_2	ta_3	ta_4	ta_5	LR	BV	SV	BR
P_1	870	810	440	200	120	50	80	0.54	4.71	3.82	2.57
P_2	860	850	480	220	120	50	100	0.56	4.47	3.56	3.91
P_3	920	890	510	250	120	50	100	0.57	4.46	3.55	4.23

Hypopygium (Fig. 6E, F). Tergites IX without anal point. Tergites IX with 23 setae. Gonocoxite 200 μm long. Superior volsella tongue shape with small projection, 50 μm long. Inferior volsella sclerotized, finger-like, 30 μm long. Gonostylus 75 μm long, with one megaseta, 10 μm long. HR 2.67; HV 2.80.

Female and immature stages unknown.

Etymology. The species is named '*zhengi*' after Prof. Le-Yi Zheng, for his outstanding contribution to the knowledge of insect taxonomy in China; noun in nominative case.

Key to the known adult males of *Boreoheptagyia* Brundin from China

1 Antenna with 13 flagellomeres **2**
– Antenna less than 9 flagellomeres **6**
2 Alula with 3–4 setae. Gonostylus short and inflated, with very short, narrow apical part ..... ***B. alulasetosa* Makarchenko, Wu & Wang**
– Alula without setae. Shape of gonostylus different **3**
3 Dorsocentrals only in single anterior group on scutum **4**
– Dorsocentrals in anterior and posterior groups on scutum **5**
4 Prealars 12. Inferior volsella with some distal teeth ..... ***B. ambigua* Makarchenko, Wu & Wang**
– Prealars 1–4. Inferior volsella without teeth ..... ***B. xinglongiensis* Makarchenko, Wu & Wang**
5 Prealars 17–28 ..... ***B. brevitarsis* (Tokunaga)**
– Prealars 10 ..... ***B. similis* (Chaudhuri & Ghosh)**
6 Antenna with 8–9 flagellomeres ..... ***B. joeli* Makarchenko**
– Antenna with 6–7 flagellomeres 7
7 Wing developed, antenna with 7 flagellomeres ..... ***B. zhengi* Lin & Liu, sp. nov.**
– Wing reduced, antenna with 6 flagellomeres ..... ***B. tibetica* Makarchenko, Wang & Willassen**

Discussion

Morphological characters of *B. kurobebrevis* from China fit well with the original description by Sasa and Okazawa (1992) and Makarchenko et al. (2008), but there are a few differences in numeric measurements: total length (2.95 mm), AR 0.82 and scutellum with 46 setae in Chinese specimen, as compared with total length 3.34 mm, AR 0.64 and scutellum with 50 setae in Japanese specimen (Makarchenko et al. 2008).

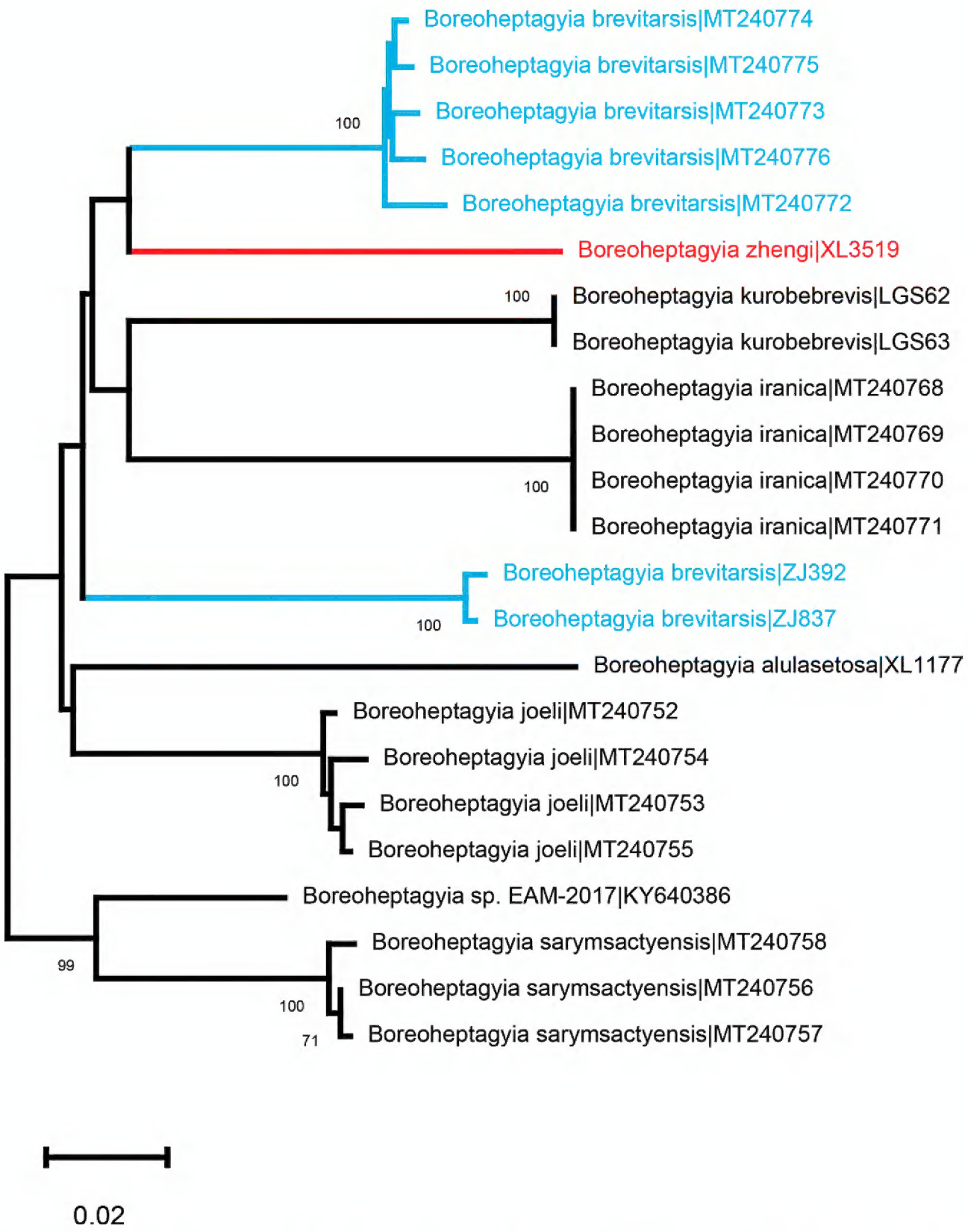

Figure 7. Neighbor-joining tree for six species of the genus *Boreoheptagyia* based on K2P distance in DNA barcodes. Numbers on branches represent bootstrap support (>70%) based on 1000 replicates; scale equals K2P genetic distance.

The new species can be easily separated from other related members of the genus by the following combination of morphological characters found in the male adult: antenna with seven flagellomeres; wing membrane covered with macrotrichia on almost the entire surface except a bare spot near the anal lobe. *Boreoheptagyia zhengi* sp. nov. keys out close to *B. tibetica* from which it can be separated in having: 1) antenna with seven flagellomeres in *B. zhengi* Lin & Liu, sp. nov., whereas the latter has six flagellomeres; 2) well-developed anal lobe in the new species and the wing membrane with microtrichiae on almost the entire surface except for a bare area near the anal lobe,

whereas *B. tibetica* has a reduced anal lobe and wing membrane with macrotrichia on the entire surface; 3) differing number of chaetae on thorax: (acrostichals 27, dorsocentrals 14 in two rows, prealars 5 in the new species) compared with (acrostichals 14, dorsocentrals 6, prealars 15–16 in *B. tibetica*).

The neighbor-joining tree based on COI DNA barcodes of *Boreoheptagyia* revealed nine distinct genetic clades (Fig. 7). The new species *B. zhengi* sp. nov. separates from *B. brevitarsis* by more than 11% divergence in COI barcodes (Fig. 7; Table 1). In addition, there are two genetic clades of *Boreoheptagyia brevitarsis* (Fig. 7), indicating that a potential cryptic species or misidentification. A further integrative taxonomic study on *Boreoheptagyia brevitarsis* is needed when the more public vouchers are available to access.

Acknowledgements

Financial support from the National Natural Science Foundation of China (31801994, 31900344, 31672264, 31672324), the China Postdoctoral Science Foundation Grant (2018M640227) and the Natural Science Foundation of Tianjin (18JCQNJC14700, 18JCYBJC96100, 20JCQNJC00420) is acknowledged with thanks.

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